Three-dimensional digital template atlas of the Macaque brain

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Abstract

We present a new three-dimensional template atlas of the anatomical subdivisions of the macaque brain, which is based on and aligned to the MRI dataset and histological sections of the Saleem and Logothetis atlas (*Saleem and Logothetis, 2012*). We describe the creation and validation of the atlas that, when registered with macaque structural or functional MRI scans, provides a straightforward means to estimate the boundaries between architectonic areas, either in a three-dimensional volume with different plane of sections, or on an inflated brain surface (cortical flat map). As such, this new template atlas is intended for use as a reference standard for macaque brain research. Atlases and templates are available as both volumes and surfaces in standard NIFTI and GIFTI formats.

Introduction

Neuroanatomical atlases are an essential aspect of studying the brain, its anatomical and functional organization, and connections. Conventional atlases, which have guided neuroscientists for more than 100 years, consist of drawings or photographs, typically from histological sections stained for cell bodies (Nissl and Golgi method), or myelin fibers (Ramón y Cajal 1899; Brodmann 1909; Kluver and Barrera 1953; See also Garey 1994). Classical anatomists have constructed atlases by magnifying such sections and drawing both major divisions of the brain as well as more subtle distinctions between brain regions based on cytoarchitectonic appearance or myelination that were evident upon staining (Brodmann 1909; Vogt and Vogt 1919; von Bonin and Bailey 1947). In the last few decades, neuroanatomical atlases have taken the form of large books of magnified photographs of stained sections combined with corresponding outline drawings that depict the precise stereotaxic coordinates of internal brain structures.

The human capacity to infer three-dimensional structure from a sequence of images is limited because of the challenges posed for understanding the topological layout of the brain using a series of two-dimensional sections. The cerebral cortex, for example, is a highly convoluted structure whose many folds result in nearby structures on a section to be located far away from one another in cortical space. It is thus often difficult to gain a perspective on the tiling of the cortical sheet by different functional areas by comparison with a conventional atlas. This problem is more severe when the angle of sectioning of a brain specimen (or block) does not precisely match the angle of sectioning in the reference atlas. To address these issues, investigators have previously taken the approach of physically flattening brain specimens, after

first separating the cortical mantle from the underlying white matter (Olavarria and Van Sluyters 1985; Tootell and Silverman 1985; Hackett et al., 1998; Sincich et al., 2003). More modern approaches allow for the virtual extraction of cortical surfaces and volumetric brain components from digitized brain scans, which one can then manipulate and analyze on a computer (Drury et al., 1996; Van Essen et al., 2001).

Within the human functional imaging community, researchers have come to rely on standard methods to align individual brains into a common anatomical space. These registration methods can employ, for example, affine transformations that apply twelve parameters to reshape the large-scale geometry of the brain, or nonlinear methods that use thousands of parameters to locally optimize the correspondence between different types of scans or scans coming from different subjects. A common practice is to align subject-specific data to a common reference space such as Talairach or MNI space, defined by the geometry of a single template brain. Despite the inherent variability across subjects, this general approach has proven useful for comparison across subjects, scanning sequences, and laboratories. It also provides a means to identify the specific brain areas associated with functional MRI responses or anatomical features of interest.

Likewise, accurate registration to a three-dimensional anatomical standard is an important capability for neuroscientists using the macaque model, which relies increasingly on the use of MRI methods. Macaque researchers need to determine the areal location from both anatomical and functional MRI scans. For functional scans, this is important, for instance, when attempting to understand the correspondence between functionally defined areas (e.g. “face patches” [Tsao et al., 2008a, b]) and histological areal boundaries. For anatomical scans, areal information is critical for targeting sites for electrophysiological experiments, anatomical tracer

injections, or local pharmacological manipulations. Using a 2D atlas for this purpose typically requires comparing an MRI dataset with labeled histological sections from one plane (Wu et al., 2000; Paxinos et al., 2009). The Saleem and Logothetis (2012) atlas offers three planes, with its areal boundaries initially identified in horizontal and coronal histology sections with different staining methods, and then interpolated into sagittal MRI sections in the same animal. However, what is really needed is a high quality 3D volumetric atlas that can be automatically computer-registered to the 3-D anatomical or functional scan from any animal, and thus used to specify the areal designation relative to experimental locations of interest. At present, there exist several options for generating 3D macaque brain templates for mapping architectonic areas onto the macaque brain: 1) *Surface based atlases*, which are based on registering published parcellation schemes or neuroanatomical data onto a standard brain (Van Essen et al., 2012; Bezgin et al., 2012; see also Rohlfing et al., 2012 and their Table 1 for the overview of some of the publicly available MRI based atlases of the non-human primate brain), 2) *Probabilistic atlases*, which rely on MRI-based parcellation but have the advantage of estimating areal boundaries based on population data (McLaren et al., 2009; Quallo et al., 2010; Frey et al., 2011; Woods et al., 2011), 3) *2D histological datasets*, which consist of stained, high-resolution histological sections and coarse labeling, but no detailed delineation of areal boundaries (Mikula et al., 2007; see http://brainmaps.org), 4) *Slice based 3D atlases*, in which 2D histological slices are converted into a 3D volumes without reference to the native MRI-based geometry (Chakravarty et al, 2009; Modha 2009), and 5) *Diffusion tensor MRI atlases* based on multiple postmortem rhesus macaque brains (Calabrese et al., 2015).

In the case of the Saleem and Logothetis atlas, the histological boundaries were carefully determined in two hemispheres of one experimental animal (D99), and from the outset the atlas

was registered to the same animal's *in vivo* high-resolution anatomical MRI scan thus preserving the native geometry of the brain. Here we present a new high-resolution 3D template atlas based on the data from D99, including anatomical parcellation of cortical, and subcortical areas (claustrum, subregions of the basal ganglia, amygdala, and hippocampal formation). We demonstrate the general utility of this digital atlas by applying it to multiple subject macaque brains, and test its validity by comparing the areas estimated from the atlas to histological sections from the same subjects. In addition to the template atlas itself, we outline a number of steps involved in the conversion and digitization of the 2D atlas into 3D template form, and its application to projects that involve anatomical, functional, or connectional imaging.

Materials and methods

The starting point for the 3D reconstruction was the published Saleem and Logothetis atlas of the macaque brain (Saleem and Logothetis 2012). The architectonic subregions of different cortical and subcortical areas from each two dimensional drawings of the sections (2D-atlas) were digitized into a 3D volume of labels. A surrogate anatomical MRI volume of better gray/white matter tissue contrast and higher spatial resolution was created by registering a high quality *ex vivo* MRI scan of a perfused brain to the original atlas space defined by the native geometry of the original MRI scan from the atlas. The 3D volume of labels was then processed with anatomically constrained interpolation to match the surrogate MRI volume, followed by manual editing to remove any residual mismatch and artifacts. Finally, the accuracy and usability of the 3D template atlas was demonstrated by applying the atlas data to a range of macaque brains of different sizes, and functional imaging data. The following sections outline these methodological aspects in the creation and testing of the atlas in greater detail.

Original 2D-atlas and MRI data

The atlas data set used in this study consists of 30 labeled sagittal slices of the left hemisphere of a male rhesus monkey (*Macaca mulatta*; case D99; 4 years old; weight 4.9 kg), and was obtained from the Saleem and Logothetis atlas (e.g., Fig. 1A; For complete set of all 30 sagittal slices, see Chapter 5 in this atlas). These slices, spaced 1mm apart were converted into 30 two-dimensional vectorised images with each region defined as a filled polygon of a different color (Fig. 1B). Canvas14 (ACD Systems, Inc.) .cvx file format was used to store those vectorised regions for each sagittal slice. The procedure for determination and coloration of each region is described in detail below.

The original MRI data set consisted of a structural brain volume of the same subject, case D99. It was acquired using a Modified Driven Equilibrium with Fourier Transform (3D-MDEFT) method using 4.7 T scanner with a 40 cm diameter bore. The scan achieved an isotropic voxel resolution of 0.5 mm^3 with dimensions of 256 X 256 X 240 voxels. This structural image was used as a basis for the construction of our 3D digital atlas (e.g., see Fig. 1A, left).

Creation of an ex-vivo surrogate anatomical volume

A structural brain image of another macaque (case DB58, male, 5.95 kg, 4.5 year old) was obtained *ex-vivo* using a magnetisation transfer ratio (MTR) sequence, which gives a T1 like contrast with high spatial resolution (250 μm isotropic), and a better signal to noise ratio (SNR > 50) (Fig. 2B). The resulting volume was then non-linearly registered to the original anatomical T1 D99 volume from the Saleem and Logothetis atlas (Fig. 2A) using Ezys image registration software (Gruslys et al 2014). Normalized Mutual Information (NMI) was used as a similarity measure. NMI was evaluated and summed in both source and target coordinate systems while

constrained diffeomorphic forward and backward transformations were simultaneously optimized in order to avoid registration biases. This step effectively allowed us to extract the prior information about sharpness of cortical boundaries from the MTR image and use it to increase D99 sharpness without affecting its shape. We adopted this newly transformed volume as *"Surrogate D99"* (Fig. 2C) used for the atlas reconstruction described below. The very high spatial resolution of the surrogate brain, along with the excellent gray/white tissue contrast, render this MRI volume much more suitable to register with other brains than the original D99, while preserving the original geometry of D99 (see Suppl Fig. S1). The perfusion-fixation protocol, and other preparations for data acquisition and scanning of the ex-vivo brain are described in detail in the previous study (Reveley et al., 2015)

Creation of 3D digital atlas template from 2D atlas

The conversion of a section-based atlas into a three-dimensional format with unique digital identifiers for each area involved a sequence of manual and automated processing steps. These steps are outlined here (see Fig. 3):

(1) Manual filling of identified areas on each section. Each named area from the 2D atlas was assigned a unique color label in vector-based CVX format. Then, based on this assignment, each area was filled in manually in each of the 30 sagittal slices of the target hemisphere using Canvas program (v14.0, ACD Systems, Inc.). The colored sagittal images were rasterized and stacked to create a coarse 3D volume of the brain (Fig. 3A). Because the initial gap between sections in the atlas was 1 mm, a simple interpolation would lead to a jagged and irregular representation of areal boundaries in both coronal and horizontal planes of sections (for e.g., see

coronal slices in Fig. 3A). To overcome this, we performed interpolation with the context of our very high-resolution surrogate anatomical volume, as described next.

(2) Affine and nonlinear section alignment. Assembling the anatomical volume from colored diagrams began with optimizing alignment to the surrogate anatomy volume (Fig. 3B). This process involved affine transformation based on a combination of manual landmarks, followed by minor non-linear adjustments subject to a NMI cost function. This step produced a coarse map of areal boundaries in the frame of the surrogate volume. However, at this stage of processing the color labels were jagged and not restricted to the gray matter boundaries (e.g., arrows in Fig. 3B), largely due to the 1 mm spacing between sections in the original data. The next step thus involved interpolation and masking.

(3) Interpolation of colored maps within gray matter mask. To create continuous labeling of areal colors that were restricted to the gray matter, we first extracted a mask of the gray matter from the high-resolution surrogate MRI volume and then performed a mask-constrained interpolation based upon the aligned colored sections from (2) above. During this procedure, cortical voxels lying outside of the gray matter mask were discarded, and unlabeled voxels within the gray matter were assigned a value based on their nearest 3D neighborhood (Manhattan distance was used as metric). This procedure led to a complete labeling of gray matter with no intrusion into white matter regions (compare Fig. 3C with 3B). However, following this step, close section-by-section inspection of the interpolated gray matter maps revealed that some labels did not adequately match the known anatomical boundaries. In some cases areal boundaries remained jagged or appeared as islands, whereas in others an area appeared to invade the wrong cortical space. Analysis revealed that these errors were not present in the originally 2D labeled diagrams and were thus an artifact introduced during the 3D construction. The next steps

were applied for the purpose of removing such artifacts and thus optimizing the accuracy of areal boundaries in the 3D volume.

(4) Assignment of cortical boundaries based on radial paths. Due to the various inaccuracies encountered when delineating the original label set on 2D slices, labels do not match perfectly when those slices are merged into a 3D volume (Fig. 3A). We reasoned that the quality of the dataset can be improved by exploiting prior information about qualitative properties that cortical regions should satisfy. Namely we assume that all cells distributed along "radial" paths should belong to the same cortical region. We call paths "radial" if they go along thickness of cortex connecting both cortical surfaces while intersecting with those surfaces at right angles. Radial paths should be straight lines connecting cortical surfaces in the regions where cortex is perfectly flat or in the regions where sulci and gyri share the same radius of curvature; in this case radial paths would also be the shortest paths connecting cortical surfaces. Still, paths are allowed to be curved in the regions where centers of curvatures of pial and WM surfaces are different, but the curvature has to be as small as possible (Fig 3De), as straight lines may not be able to be normal to both cortical surfaces at once (Fig 3Db). Please note that we cannot simply define radial paths to be always straight and normal to one of the cortical surfaces as we would not cover all cortical volume (Fig 3Dd) or would intersect and hence cover some regions multiple times (Fig 3Dc) which would make enforcement of the same label identifier along the path ill-defined. It could also be argued that choosing one of the cortical surfaces as "special" is not be a very elegant solution, as this might introduce different biases in the regions of sulci and gyri.

We designed an algorithm to find radial paths to be symmetric in its treatment of both cortical surfaces while producing a set of non-intersecting paths densely covering all cortical

volume (See next paragraph for more detailed description). We also designed another algorithm, which made sure that all points lying on any given path were assigned the same (most frequent) label. This helped us to avoid the incorrect situation that different layers of the same cortical columns would be assigned to different cortical areas while also significantly increasing smoothness of cortical region boundaries (Fig 3C).

We used non-intersecting radial paths within the cortical thickness connecting pial and white matter surfaces that allowed us to constrain areal assignments within the high-resolution gray matter mask. This procedure ensured that cortical boundaries always followed a radial direction through the cortex. This process began with an assignment of a vector defining path direction on every point of the grey matter volume. Vectors were assigned such that paths produced by integrating (following) vector directions at each point connected pial and WM surfaces without forming any loops or path intersections (e.g the vector field was constructed to be curl-free and divergence-free anywhere but on the cortical surfaces). Vectors can be interpreted as defining fluid velocity at each point and paths can be interpreted as streak lines connecting cortical surfaces. One simple way allowing us to find a vector field satisfying all the desired properties was to solve Laplace's equation by setting boundary conditions as +1 and -1 on both cortical surfaces (Fig 3De). Laplace's equation was solved on a finite grid with one point per voxel. A gradient of the resulting potential produced a vector field, and integrating the vector field produced paths densely covering all of the grey matter and satisfying all the desired properties, namely: intersecting both cortical surfaces at right angles, densely covering all cortical volume, forming no loops, not intersecting each other, producing straight paths where cortical surfaces are flat and straight radial paths where the cortical surfaces share the same center of curvature. Each path was assigned a region identifier by majority voting of the voxels

that any given path crossed. Each point in a cortical 3D space was allocated a region identifier defined by the only path that crossed that point. As voxels are of a finite size, region identifiers for each voxel were estimated by a majority voting of all the points inside. Further details about these procedures can be found in *Gruslys A, Development and Applications of GPU Based Medical Image Registration, PhD Thesis, University of Cambridge, 2014.*

In summary, this method allowed us to ensure that all voxels within any given cortical column were assigned the same region label.

(5) Removal of residual islands and fine labeling. In the final step it was necessary to remove a small number of isolated clusters or *"islands"* of areal labels that were spatially separated from the main area. The existence of such islands reflects, in part, the inherent difficulty in assigning strict areal boundaries based on cytoarchitectonic, and, in part, residual errors associated with the processing steps above. We removed these islands if they were less than one quarter the size of the parent region by filling them in according to the neighboring areal assignments. Following these steps, the labeled surrogate brain (Fig. 3E-F) exhibited complete labeling of region restricted to the cortex, with clear, radial divisions between adjacent cortical areas.

(6) Final delineation, verification, and mirroring of architectonic areas. Following the above steps of construction, interpolation, masking, and artifact correction, the 3D dataset was integrated into the AFNI (Analysis of Functional NeuroImages; Cox, 1996), and SUMA (Surface Mapper; Saad et al., 2012) software packages and saved in GIFTI and NIFTI formats, respectively. The dataset was then subject to a series of manual verification and correction of areal extent and architectonic borders of different areas in comparison with the original atlas

sections from Saleem and Logothetis atlas (2012). These manual corrections were made on the right side of the brain and were mirrored to create a symmetric brain. We applied an additional novel smoothing algorithm to find the mode within a local spherical neighborhood, in this case 0.5 mm. Some regions with exceptionally fine features, like the lenticular bridges of the striatum, did not survive modal smoothing. In these cases - claustrum, striatum and several hippocampal structures, the datasets were manually modified to maintain their three dimensional integrity and smoothness. The accuracy of the reconstruction method was then verified by comparing the resliced volume to the original section diagrams. This 3D digital volume (Fig. 4) is presently available in the AFNI and SUMA analysis packages to register and apply to the brains of other individual macaques, in order to serve as a guide for any of a number of research applications for which accurate knowledge of areal boundaries is desirable (e.g. see Fig. 5 in Results).

(7) Establishment of 3D Atlas coordinate system. Based on its utility for MRI navigation, the 3D atlas coordinate system was defined to have its origin (0,0,0) at the anterior commissure (AC). A simple affine transformation can be used to transform coordinates between this AC coordinate system, which is convenient for neuroimaging, and the stereotaxic ear bar zero (EBZ) coordinate system of the original sections in the Saleem and Logothetis atlas (Fig. 4F). In both AC and EBZ based coordinate systems, all horizontal slices were aligned parallel to the horizontal plane passing through the interaural line and infraorbital ridge, and coronal slices were aligned orthogonal (perpendicular) to horizontal plane. Note that in contrast with other templates (e.g., Calabrese et al., 2015), the AC and posterior commissure (PC) was not used here to define the slicing angle.

Anatomical and functional MR scanning of test cases in this study

High contrast anatomical scans. In six normal, and healthy animals (age: 1.2 to 14.8 years; weighing between 2.55 to 5.5 k.g.), MR anatomical images were acquired in a 4.7 T horizontal scanner (Bruker Biospec 47/40) using a modified driven equilibrium Fourier transform (MDEFT) method. The monkey was anesthetized with isoflurane and placed into the scanner in a sphinx position with its head secured in a holding frame. A single loop circular coil with a diameter of 14 to 16.5 cm was placed on top of the animal's head. The whole-brain MDEFT images were acquired in a 3D volume with a field of view 96 x 96 x 70 mm^3, and 0.5 mm isotropic voxel size. The read-out had an 11 ms repetition time, a 4.1 ms echo time, and a 11.6 degree flip angle. The MDEFT preparation had a 1240 ms pre-inversion time, and a 960 ms post-inversion time for optimized T1 contrast at 4.7 T. Each 3D volume took 25.5 min to acquire without averaging. Most of the scans were acquired with 2 averages and took 51 min. These cases were illustrated in Fig. 5. Similar anatomical scans were obtained in two other cases illustrated in Figs. 6 and 7.

Awake functional scans. In one monkey, we carried out awake functional scanning in repeated sessions. The contrast agent MION (magnetic iron oxide nanoparticles) was injected into the saphenous vein prior to each session. The animal was shown alternating blocks of faces and scrambled faces, with the results shown registered to the 3D atlas in Fig. 8. Further methodological detail about the functional MR scanning is provided in Russ and Leopold (2015). All procedures were approved by the Animal Care and Use Committee of the United States National Institutes of Health (NIH), National Institutes of Mental Health, and followed NIH guidelines.

Creation of flat map registered to test case

The flattened cortical surfaces were created using a combination of AFNI (Cox 1996) and Caret software (Van Essen et al., 2001). Specifically, the animal's high resolution anatomical MRI was skull stripped and then the image intensities were normalized using the AFNI software suite. The resulting anatomical files were then imported into Caret software, where a set of surfaces was created from a white matter mask. The white matter mask was created by first using the automated gray/white delineation tools in Caret, and then manual fixing any errors in white matter selection in all three planes of sections. The white matter mask from each hemisphere was then used to create a set of surface maps and a flattened cortical surface following the procedures outlined in Van Essen et al. (2001). The surface maps and their related anatomical volumes were then exported back into AFNI, where the digital D99 atlas was registered to the exported volume.

Results

Registering identified areas from 3D atlas to range of test subjects

The 3D atlas is of great use in its application to projects that involve anatomical, functional, or connectional imaging. In particular, it is of immediate value to register the 3D atlas to a given macaque subject's brain in order to determine the areal location of fMRI responses, spatial distribution of labeled neurons or terminals in different cortical areas after the anatomical tracer injections, or electrophysiology recording sites. While less complex than in the human, the macaque cerebral cortex has a number of sulci that might pose difficulties for registration. As macaque brains vary to some extent in their sulcal patterns, one test of the utility of the present atlas is whether it can be registered to a range of different macaque subjects. To this end, we developed a novel macaque processing pipeline within AFNI and SUMA to optimally register

the atlas to T1 MRI images of individual macaque brains (see below). This procedure involved a sequence of affine and nonlinear registration steps. An initial affine step gave an approximate scaling and rotation to the template. The affinely warped subject brain was gradually warped to the template by progressively smaller nonlinear warps. This procedure resulted in the subject brain data in register with the D99 template space. By inverting the combination of affine and nonlinear transformations, the atlas segmentation was warped to each subject's original native space. The results of this pipeline are shown in Fig. 5 for six monkeys of different genders, ages, and sizes. While determining the precise matching between the determined areas and the histologically determined regions of all six animals is a large project that is beyond the scope of the present report, these results demonstrate that a straightforward affine and nonlinear warping is sufficient to provide atlas-based estimates of areal boundaries on macaque subjects *in vivo*.

Validating 3D areal registration: comparison to the printed atlas

A major component of validating the accuracy of the 3D atlas involved the comparison of its slices with the original Saleem and Logothetis atlas (Figure 6A-C). In this example, we first registered the MRI volume of the subject MQ to the 3D digital atlas (A), or vice-versa (B), and then compared the selected slices from these volumes to corresponding original atlas sections (C). Subject MQ was selected because histological data from this animal was also available, as described in the next section. As expected, the registration of subject MQ to the digital atlas (original native space) led to areal labeling that closely resembled that in the Saleem and Logothetis atlas (compare the right hemisphere in A and C). In the case that the digital atlas was registered to the brain of subject MQ, thus retaining that native geometry obtained during the MRI of MQ, the areas again matched closely (compare B and C). It should be noted that in order

to align the MRI with the histological sections described in the next section, we slightly rotated the volume of MQ in the dorsoventral plane around the mediolateral axis. As a result, there is an expected difference in dorsal and ventral regions, reflecting the cutting angle of the histological slice, which was not strictly on the coronal plane. Dorsally, this angle results in a shift relative to the border between anterior and posterior cingulate gyrus (e.g., subregions of area 23 in A but 24' in B). Ventrally, the angle results in a shift relative to the border between anterior and posterior TE in the inferotemporal cortex (e.g., area TEad in A but both areas TEad / TEpd in B). These cortical regions are indicated by red stars in both A and B. Finally, we note that in Fig. 6B the digital atlas is registered to subject's original native space, which is desirable for many applications, thus the precise sulcal geometry of the slices appear somewhat different from those in the atlas (i.e. in contrast to Fig. 6A and C).

Validating 3D areal registration: comparison to histological sections

In two subjects, we demonstrated accurate matching of brain areas determined by MRI registration with those identified using the cytoarchitectonic analysis of histological sections from the same brains (Fig. 7). Here the D99 digital template atlas was registered to the T1 MRI volume of two individual brains (cases MQ and BASS), which were different from the cases used in Figure 5. We matched cortical and subcortical areas in the coronal slices of registered brain volumes (Fig. 7, arrows in E-H) with the corresponding histology sections, stained for either neurofilament protein (SMI-32 staining) or the Nissl substance (Fig. 7 M-T). The architectonic features of these selected areas were previously identified in both staining methods described here (Saleem et al., 2007; Saleem and Logothetis, 2012; Scott et al., 2015). Sections stained for SMI-32 reveal different laminar distribution of pyramidal neurons in cortical areas, and subcortical regions of the macaque monkey (Hof et al., 2004; Saleem and Logothetis 2012).

Atlas labeling of the primary auditory area A1, medial auditory belt area RM, medial temporal pole area TGdd, medial temporal lobe regions (entorhinal and CA1 regions of the hippocampus), and certain subcortical structures, including subregions of the basal ganglia (SN and STN), and caudal hypothalamic area, the mammillary bodies (MB) (Fig. 7, M-T) closely matched the areas we identified histologically in our previous study (Saleem and Logothetis, 2012; Scott et al., 2015, for e.g., see their Fig. 3F, I, Q).

We also demonstrated the general utility of registering 3D atlas to the experimental (fMRI) data in an additional test subject (Fig. 8), described in the following section.

Applications for functional MR imaging

The most pressing application of the 3D template atlas derived from the Saleem and Logothetis atlas is likely to be the systematic identification of areas associated with fMRI activation patterns. Of particular interest is the spatial relationship between the histologically identified cortical areas catalogued in the atlas and functionally defined regions associated with a particular stimulus or task. An example of this application can be seen in Fig. 8, which uses the 3D digital template atlas (D99) to study the anatomical location and extents of face patches. The fMRI-defined face patches were in this case derived by contrasting the responses to intact versus scrambled faces presented in a block design. Here we focus on the anatomical location of the anterior lateral (AL) and middle fundus (MF) face patches in the temporal cortex (Fig. 8A), as defined by Tsao et al. (2003). A direct comparison of the functional map with the digital atlas sections (Fig. 8B), and then to inflated or flattened cortical surface (Fig. 8C), demonstrates that the MF face patches occupy a restricted longitudinal position of a cytoarchitectonically-defined area IPa at the fundus of the STS, whereas the AL face patch occupies a broader region

straddling the boundary between areas TEm and TEad at the ventral lip of the superior temporal sulcus (STS) (Fig. 8B-D). In addition, one can see the ML face patch in TEm, the PL face patch in TEO (or PITd), and the small AF face patch straddles the border between IPa and TEa (Fig. 8C). The activity visible in area V4 is a consequence of the specific contrast used here (intact vs. scrambled faces).

Implementation and Distribution

Atlases and templates are available as both volumes and surfaces in standard NIFTI and GIFTI formats. While this 3D digital atlas can be used in different image registration and analysis software packages, here we use the AFNI program with its advanced atlas features for purposes of demonstration.

The 3D template volume and atlas are now available for download through the AFNI and SUMA website, at *http://afni.nimh.nih.gov/pub/dist/atlases/macaque*. The atlas is integrated into the most recent versions of AFNI and SUMA, making for straightforward identification of areal identity in any macaque subject registered to the template and for the individual macaque subject in its own native space by the inverse transformations. As in our example macaques, standard alignment programs within AFNI can be used to align macaque subjects to the D99 template space. A script has been made available at the same link above. The script includes several steps: First it implements affine alignment to the D99 template followed by a nonlinear alignment, and then it inverts the combination of both the affine and nonlinear transformations to transform the atlas segmentation into the original native space of the data. Because the atlas segmentation is provided within the header of the dataset, the AFNI and SUMA software can provide on-the-fly labeling information about each individual macaque to the user via its "*whereami"* interface.

This functionality can be accessible through the AFNI GUI or scripted through the command line interface. Within the AFNI program, the whereami interface provides further information for the equivalent coordinate in the book version of the Saleem-Logothetis atlas. The book version uses an ear-bar zero (EBZ) coordinate origin while the distributed template uses an AC (anterior commissure) as the coordinate for the origin. By using a simple affine transformation that defines the shift between the two coordinate systems, the corresponding book EBZ and the dataset's AC-based locations are determined on-the-fly.

Surfaces generated for the template and atlas regions can be viewed within SUMA. Atlas region surfaces, generated using SUMA's IsoSurface program, are distributed with the atlas. Interprocess communications between AFNI and SUMA enable surfaces and volumes to be linked together; clicking on a volume or a surface will update the crosshair in the other viewer with a concomitant change in the labeling information in the whereami and image viewers. The SUMA software can show each of the region surfaces with each surface individually controllable for transparency and mesh display. This capability allows for more of a fly-through type of control where regions can be "peeled" away to reveal underlying regions.

Discussion

The present macaque digital template atlas, derived from the Saleem and Logothetis atlas, is one of several digital monkey atlases that have been created in recent years (McLaren et al., 2009; Modha 2009; Woods et al., 2011; Quallo et al., 2010; Frey et al., 2011; Van Essen et al., 2012; Bezgin et al., 2012). In the following paragraphs, we briefly compare among atlases, highlighting advantages of the present offering.

D99 digital atlas versus other atlases

Perhaps the most important unique feature of the present atlas is the strict adherence to an MRI scan from the same brain. In the two-dimensional atlas, this aspect was critical for maintaining the native *in vivo* geometry of the brain during the establishment of areal boundaries based on histological sections. The printed atlas thus provided a 3-D stereotaxic coordinate system that was accurately shaped by the anatomical MRI volume of the original brain. This unique feature of the Saleem and Logothetis atlas is perhaps even more important in the transformation to the digital template atlas here. In this case, the well-specified geometry of the original animal allows one to accurately register areal boundaries onto other macaque brains. Moreover, this is done in a straightforward manner using widely available tools of whole-brain MRI registration. Furthermore, the present digital template atlas replaces the original anatomical volume with a high resolution, high contrast surrogate anatomical volume that has itself been registered carefully to the *in vivo* MRI volume of the brain used to construct the original Saleem and Logothetis atlas (Fig. 2). Preserving the known geometry of this brain has enabled the best possible estimation of histological divisions in the high quality surrogate. The surrogate, in turn, can then be used to determine the areal boundaries in experimental animals, using widely available tools that use affine and non-affine warping between MRI volumes (Chakravarty 2009). The transformation derived from this warping, when then applied to the 3D digital atlas volume, allows for as accurate as possible labeling cytoarchitectonic areas in the brains of individual animals.

This feature extends the work of previous digital atlases, such as that of Frey et al. (2011) based on the Paxinos macaque atlas (Paxinos et al., 2009). In the construction of that atlas, areal boundaries were first delineated on coronal MRI slices using histological data that was linearly and nonlinearly transformed to match a composite volume of 25 rhesus and cynomolgous

macaque brains (see also Chakravarty et al, 2008, 2009). The introduction of this new template atlas and space, the latter termed the Montreal Neurological Institute space for the macaque (henceforth "macaque MNI space") was an important step forward and offers an excellent estimate of areal boundaries given that the data were from different animals. In another approach, Modha (Modha 2009) applied a mathematical vector method to convert 151 macaque histological slices from the same atlas (Paxinos et al., 2009) into a volume, and ultimately into a surface atlas. While useful for some applications, this vector approach does not attempt to preserve the native geometry of the brain, thus the registration accuracy of the resulting volumetric or surface atlas will be always in question. The present digital atlas avoids the step of geometrically transforming the cytoarchitectonic information from histological sections to match the layout of a different animal's brain, which can introduce errors that are difficult to evaluate. Instead, the postmortem tissue sections were carefully block-face aligned to the MR image prior to analysis (Saleem and Logothetis 2012; chapter 1). As a result, the alignment accuracy between areal boundaries and gross anatomical features is optimized in the present case.

In another approach, McLaren et al (McLaren et al, 2009) used population-averaged anatomical MRI volume that was registered with the Saleem and Logothetis atlas. This step allowed for investigators to register their own data into the geometry of the animal from which the cytoarchitectonic data was originally evaluated. This type of registration then allows for a comparison of positions in the brain, relative to gross anatomical features, to be compared with the printed atlas. In theory, the reverse transformation to this space also permits precise determination of stereotaxic coordinates for individual monkeys, compensating for intersubject variation. However, the creation of such a template did not offer a digitized rendition of cortical areal boundaries, thus determining the identity of an area always required comparison with the

printed atlas. In contrast, the present digital atlas template allows for the areal specifications, themselves in a 3D volume similar to the anatomical MRI itself, to be warped onto any macaque data set using a transformation obtained from any of a large number of MRI processing packages. Thus the areal label can be brought to the raw data, or the raw data to the template atlas, in neither case requiring consultation of the printed sections.

In addition, the present digital template atlas offers a substrate for template-based group analysis, for example using the surface map derived from the surrogate volume. At present, electrophysiological and fMRI studies in the monkey have only minimally taken the approach of combining data on a common template (see Janssens et al., 2014). Defining an area-labeled template space based on the geometry of animal used for the Saleem and Logothetis offers one means for combining macaque experimental data into a common and well-annotated space. The only requirement is the acquisition of an anatomical volume, which can be used to transform data into the common template volume or surface. This 3D digital template atlas can also be integrated with the macaque connectome atlas (Saleem et al., 2015a, b) in AFNI and SUMA interface, which allow users to navigate the atlas with connectional data interactively in 3D, and integrate the information directly with their anatomical and functional imaging results in surface modes.

Generalization and validation

The compilation of any brain atlas, which includes the assignment of boundaries and names to individual areas, is an inherently imperfect endeavor whose main goal is to provide a common anatomical framework for a range of research projects and data. In the present case, the innovation rests on the creation of 3D digital macaque atlas whose anatomical borders were,

from the outset, created based on MR-registered histological sections. This digital version of the Saleem and Logothetis atlas is based on the precise histological borders from one particular monkey (D99), which because of the initial registration to the MRI from the same animal can be represented on the brain of any experimental animal. Other MRI atlases, including probabilistic (McLaren et al., 2009; Quallo et al., 2010; Bezgin et al., 2012; Janssens et al., 2014), and surface-based (Van Essen et al., 2012) atlases, generally determine areal boundaries using different methods, and may therefore draw different conclusions regarding subdivisions, large-scale organization, and nomenclature. It is important that efforts to identify and summarize the brain's structural and functional organization continue to evolve.

A thorough validation of this 3D atlas, such as estimating the architectonic boundaries between different areas for a population of macaque brains, is beyond the scope of the present report as it would be an enormous project. Nonetheless, we performed three analyses that indicate that the correspondence to experimental macaque subjects is generally good and useful. In the first analysis, we showed that the MRI registration procedure could be smoothly applied to subjects of different genders and brain sizes, thus it is possible to estimate the histological boundaries in any monkey subject (Fig. 5). In the second analysis, we showed labeling a test subject's brain using this approach yielded labeled slices that closely matched those of the printed atlas (Figure 6). Correspondence was high both for sagittal sections (used to create the 3D atlas) as well as horizontal and coronal sections (matching the D99 histological data). In the third analysis, we showed a good match between areas in a new monkey subject's labeled using the 3D digital atlas with histological sections obtained from the same animal (Figure 7).

Architectonic versus functional maps

There are multiple methods and approaches to determine areal positions across the cortical surface. The areal boundaries defined in a cytoarchitectonic atlas have historically been the most important reference scheme for identifying and naming brain regions of interest. Functional mapping, such as that achieved through fMRI localizers, is another approach. While the two methods correspond closely in some cases, such as in the early retinotopically-organized visual areas, they can be taken as largely independent measures in others, with each giving rise to its own nomenclature. For example, face patches such as AL are defined and named according to location of their fMRI functional contrast, and this functional mapping offers a useful and reliable set of reference locations in the frontal and inferotemporal cortex (Tsao et al., 2003; 2008a, b). Both measures are closely related to the brain's functional anatomy and thus their relationship is important for understanding the complex relationship between structure and function. Thus, while cytoarchitectonic boundaries are likely to remain the principal source of areal definitions in the brain, the capacity to fluidly superimpose function on such maps is of great value for the future. In the case of a labeled digital template MRI volume, the capacity to reslice, inflate, or flatten the digital brain, as shown in Fig. 8D, allows for the cytoarchitectonic boundaries to be easily estimated on individual subjects, thus permitting a systematic investigation of the relationship between the two measures of brain organization.

To summarize, we have created a new high-quality MRI template and corresponding digital atlas. The atlas provides a readily usable standard for region definition while the template provides a standard reference and space. This standard space allows for macaque research to be reported on a common basis across research sites and across macaques. Additionally, the atlas allows for automated analysis against a set of standard region locations. The current atlas,

template MRI datasets, surfaces and user scripts for aligning individual subjects to this template are publicly available in the following link. *http://afni.nimh.nih.gov/pub/dist/atlases/macaque*

It should be noted that this atlas should not be altered without prior approval from the senior author of this work.

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Figure Legends

Figure 1. *Original 2D atlas and MRI data of rhesus macaque brain. (*A) An example of two-dimensional sagittal slice with delineated cortical and subcortical areas, obtained from sagittal dataset of 30 one mm interval sections in Saleem and Logothetis (2012) atlas. The

corresponding in-vivo MRI slice, and the abbreviation list of delineated areas in the sagittal section is also shown on the left. The dorsal view of the rendered brain image on the top right indicates the mediolateral location of this sagittal slice, which is located 7 mm lateral from the midline. (B) *Examples of vectorized atlas sections.* The 30 sagittal sections were then converted into vectorised images with each region marked as filled polygon of a different color using Canvas program (.cvx file format). These 30 slices were stacked and created 3D volume of the brain using "*surrogate D99 brain"*, aligned to original MRI dataset, and sequence of manual and automated processing steps as shown in figures 2 and 3.

Figure 2. *Ex-vivo surrogate anatomical volume.* The structural image of another monkey (DB58 T1) with high spatial resolution (250 um isotropic) was obtained ex-vivo using magnetisation transfer ratio sequence (B), and was non-linearly registered to the original T1 D99 from the Saleem and Logothetis atlas (A). We used this newly transformed volume *("Surrogate D99;* C) for atlas reconstruction as shown in figure 3. Note the correspondence of sulci and gyri in both original D99 and Surrogate D99 (white arrows in A and C) but see different sulcal patterns in DB58 T1 (gray arrows in B).

Figure 3. *Creation of the 3D digital template atlas from 2D atlas sections.* **(**A) 3D volume of the brain with delineation of cortical areas, created from 30 colored sagittal images. Note the rasterized or coarse appearance of section in other (coronal) plane of sections. (B) The coarse map of the areal boundaries was registered with the surrogate volume using affine transformation and non-linear adjustments. Note that the color labels were jagged and not restricted to the gray matter boundaries (see arrows). (C) Interpolation of colored maps within the gray matter mask, obtained from high-resolution surrogate MRI volume. Note the complete labeling of gray matter with no intrusion into adjacent white matter regions (see arrows) but some labels did not

adequately match the known anatomical boundaries. (D) Assignment of cortical boundaries based on radial paths (see the methods for more detail). (E, F) The labeled surrogate brain with complete labeling of region restricted to the cortex, with clear, radial divisions between neighboring cortical areas (compare final map in F with the initial rasterised map in A). Following the construction of atlas as shown in A-F, the 3D dataset was integrated into AFNI and SUMA interface (see figure 4), where the manual correction of areal extent and architectonic borders of different regions were done in comparison with the original sections from Saleem and Logothetis atlas.

Figure 4. *3D digital template atlas in AFNI and SUMA interface.* Areal delineations of different cortical and subcortical areas in sagittal, horizontal, and coronal plane of sections (A-C), and on the 3D brain surface (D), which is based on the Saleem and Logothetis atlas (E), displayed in AFNI/SUMA window. The two different stereotaxic coordinates of current location (cross hairs; e.g., area 45a), one with reference to anterior commissure (AC), and other with reference to Ear Bar Zero in Saleem and Logothetis atlas are also indicated in AFNI "whereami" window (see "Focus point" in F).

Figure 5. *Registration of 3D atlas to various test subjects.* Here D99 digital template atlas is registered with T1 MRI images of 6 individual brains of different age groups using a novel-processing pipeline developed within AFNI and SUMA (see the text). (A) One of the sagittal slices from D99 digital atlas (+14 or +15 mm from the midline) with delineated cortical and subcortical areas. The corresponding slice is also indicated on the D99 rendered brain with MRI, created in software Mango. (C-H) Sagittal slices from six animals, with the D99 atlas registered to the MRI images of each animal in its own native space. Note the corresponding location of ventrolateral prefrontal region in the ventral bank of principal sulcus (area 46v; cross hair) in

D99 digital atlas and 6 other animals. Abbreviations: 46v, ventrolateral prefrontal area; cla, claustrum; F1, agranular frontal area F1 (or area 4); pu, putamen; TF, area TF of the parahippocampal cortex.

Figure 6. *Comparison of architectonic areas in the registered MRI volumes with the corresponding sections in Saleem and Logothetis (2012) atlas.* (A, B) The coronal slices with delineated cortical and subcortical areas in subject MQ registered to digital atlas (D99) and digital atlas registered to subject MQ, respectively. The coronal section in B is the same section as shown in Fig. 7F, which is digitally rotated in the dorsoventral plane around the mediolateral axis to match with the corresponding histology section from the same case illustrated in Fig. 7J. (C) Corresponding section drawing of the right hemisphere with delineated areas from Saleem and Logothetis atlas (see their Fig. 85, page 201). This slice is located 13 mm anterior to the ear bar zero. Note that as expected, the labeled regions in A, where the subject MQ is registered to digital atlas (to its original native space) closely matched with regions in Saleem and Logothetis atlas (compare the cortical and subcortical areas in A and C). As noted above, the registered volume in B is slightly rotated to match with the corresponding histology section (see figure 7). This resulted in few mismatched cortical areas at the border between anterior and posterior cingulate gyrus (areas 23 and 24') dorsally, and anterior and posterior TE in the inferotemporal cortex (areas TEad and TEpd) ventrally (compare red stars in A and B). See the result section for more detail.

Figure 7 *Registration of 3D atlas to different test subjects with histological confirmation of architectonic areas.* In this example, the D99 digital template atlas is also registered with T1 MRI volume of two individual brains that are different from the cases shown in Figure 5. (A-D) Selected coronal slices from in-vivo T1 weighted MRI volume of cases MQ and BASS. (E-H)

Digital atlas (D99) registered and overlaid on the MQ and BASS MRI volumes (same coronal slices as shown in A-D). (I-L) Corresponding histology sections of the left hemisphere in MQ and BASS (green rectangular boxes in A-D) stained immunohistochemically for the neurofilament protein, recognized by SMI-32 antibody (I-K), and Nissl staining (L). We digitally rotated both MRI and registered volumes to match with histology sections. Note the correspondence of sulci and gyri in both MRI/registered volume and histology sections. We also confirmed the spatial location and architectonic features of the selected cortical and subcortical areas in the registered slices (arrows in E-H; small boxes in 1st and 3rd column) with the corresponding histology sections as illustrated in M-T. (M-R) High-power photomicrographs showing the differential distribution of SMI-32 positive pyramidal neurons in the auditory (A1, RM), medial temporal lobe (EC, CA1), dorsal temporal pole (TGdd), and subcortical (STN, SN, and MB) areas. We also confirmed the spatial location, and architectonic features of auditory areas in case MQ (e.g., primary auditory area A1 and medial belt area RM) with reference to our previous study (see Scott et al., 2015, their Fig. 3F, I, Q). (S-T) High-power photomicrographs showing the architectonic features of the CA1 region of hippocampus, entorhinal cortex (EC), and adjacent areas in the Nissl stained section.

Figure 8. *Mapping fMRI results onto the digital atlas.* (A) fMRI activation from a subject depicting the location of regions responsive to faces greater than scrambled faces. Activity is displayed over the subject's high-resolution anatomical images and thresholded at $t > 10$. The top row shows the sagittal and coronal MR images displaying the location of the right anterior lateral (AL) face patch from the lower bank of the superior temporal sulcus (STS). Bottom row shows the sagittal and coronal MR images displaying the location of the right middle fundus (MF) face patch within the fundus of the STS. (B) Digital atlas (D99) registered and overlaid on the

subjects anatomical images. Each color represents the delineated cortical region in the atlas. The top and bottom rows indicate the location of the face patches (same as in A), with reference to architectonic areas. For example, AL face patch is located within the subregion of area TEm and MF is located within the subregion of area IPa. (C) fMRI activity from A projected on to the right hemisphere of the subject's flatten cortical surface. White lines represent the areal boundaries of regions throughout the temporal lobe based on the areal map in D. The locations of the face patches depicted in A (AL, MF), and other face patches (AF, ML, PL) are also indicated in the map. Note that the anterior medial (AM) face patch at the border between TEad and TEav is not visible in this case. In addition, the activity visible in area V4 is a consequence of the specific contrast used here (intact vs. scrambled faces). (D) The digital atlas projected on to the same flattened surfaces as in C. Each color represents a different cortical region. Abbreviations: AF, anterior fundus; ML, middle lateral; PL, posterior lateral. For the abbreviation of different cortical areas in D, see Saleem and Logothetis (2012) atlas.

Supple Fig. 1. *Examine the geometrical match between the surrogate brain volume and the original brain volume.* Pial surface (red contour) and white matter surface (yellow contour) derived from the surrogate brain were overlaid on both the surrogate brain (A) and the original D99 brain (B) on two selected sagittal sections. The surface contours fit to the tissue boundaries equally well on both brains.